SUPPLEMENTAL METHODS

Over-expression of MKP-3 and knockdown of MKP-3 and FOXO1 in primary rat hepatocytes

Primary rat hepatocytes were seeded as described in experimental procedures. The next day, cells were transduced with adenoviruses expressing GFP, MKP-3 or shGFP, shMKP-3, shFOXO1 at MOI 50. For RNA extraction, cells were incubated in William's E medium containing 0.5% BSA, 1 μM dexamethasone and 1 mM 8-bromo-cAMP for 8 hr forty-eight hours post infection. For glucose production, cells were incubated in William's E medium containing 0.5% BSA, 1 μM dexamethasone and 1 mM 8-bromo-cAMP for 5 hr forty-eight hours post infection, then incubated in 0.5 ml/well of phenol red-free, glucose-free DMEM containing 1μM dexamethasone, 2 mM pyruvate, 20 mM lactate and 1mM 8-bromo-cAMP for 3h. Medium was collected and subjected to glucose measurement. The glucose output rate was normalized by cellular protein content.

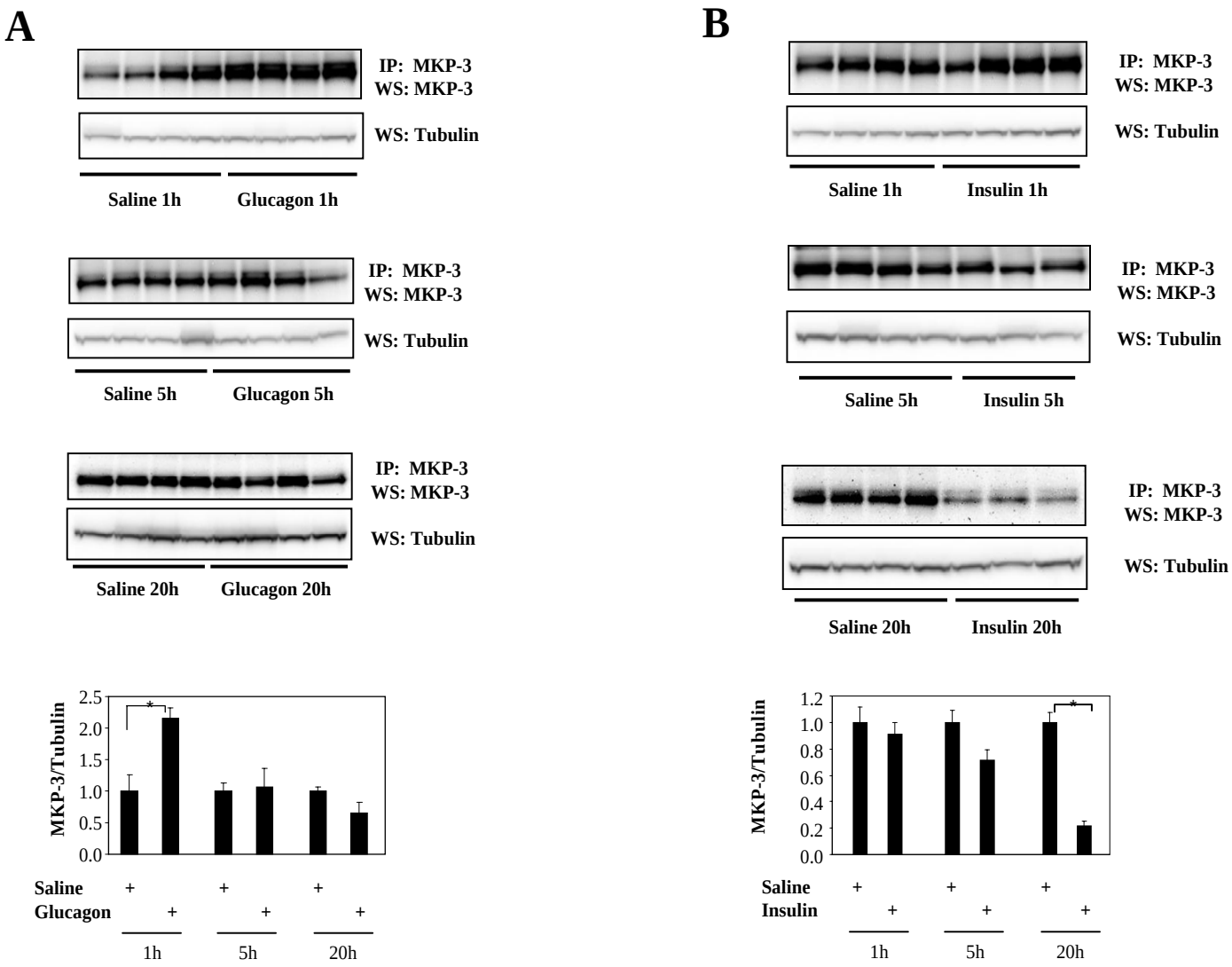
Immunolocalization

Fao cells were infected with adenoviruses expressing a control protein (an inactive kinase) or MKP-3. Twenty-four hours post infection, cells were transfected with GFP-FOXO1 expression plasmid. Forty-eight hours after infection, cells were incubated in serum-free RPMI 1640 medium in the presence of Veh, 1μM Dex or 1μM Dex plus 100ng/ml insulin overnight. Next day, cells were incubated in serum-free and glucose-free DMEM medium supplemented with 2mM sodium pyruvate and 20mM sodium lactate for another three hours in the presence of veh, Dex or Dex plus insulin. Cells were

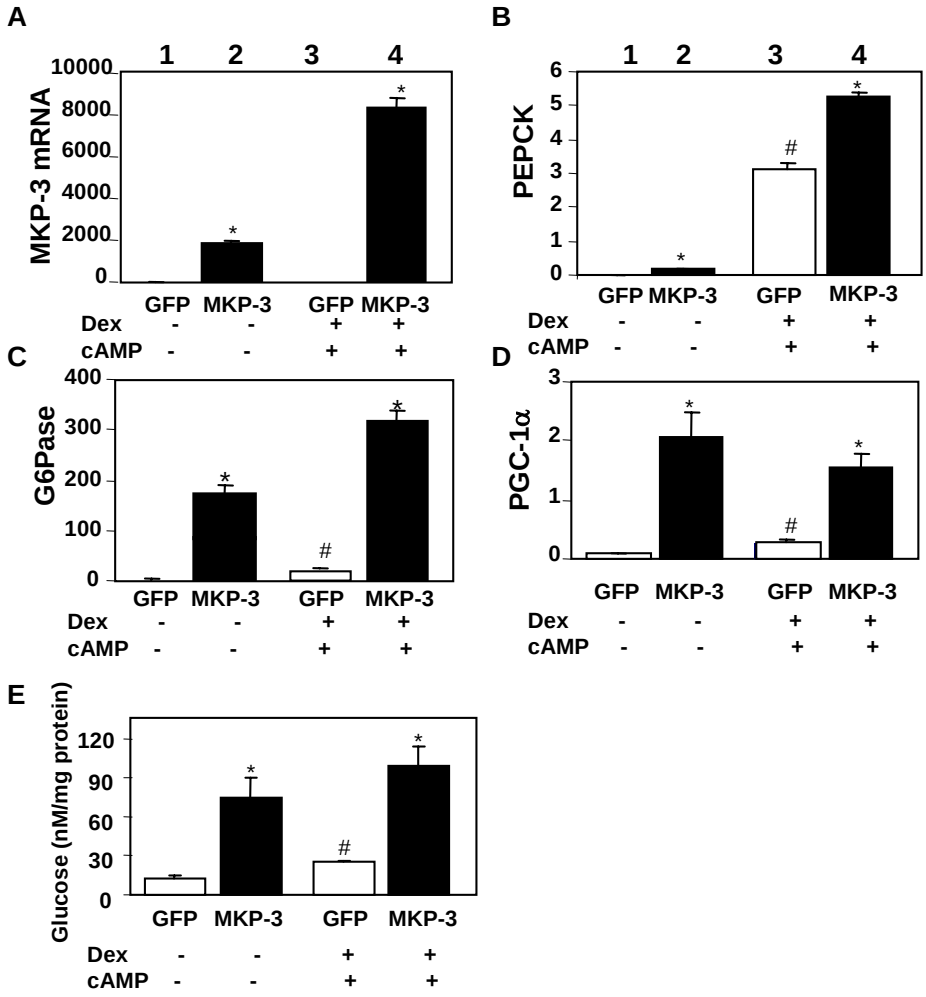
then fixed in 5% PBS-buffered formalin for 10 minutes and examined for FOXO1 localization.

Mouse model

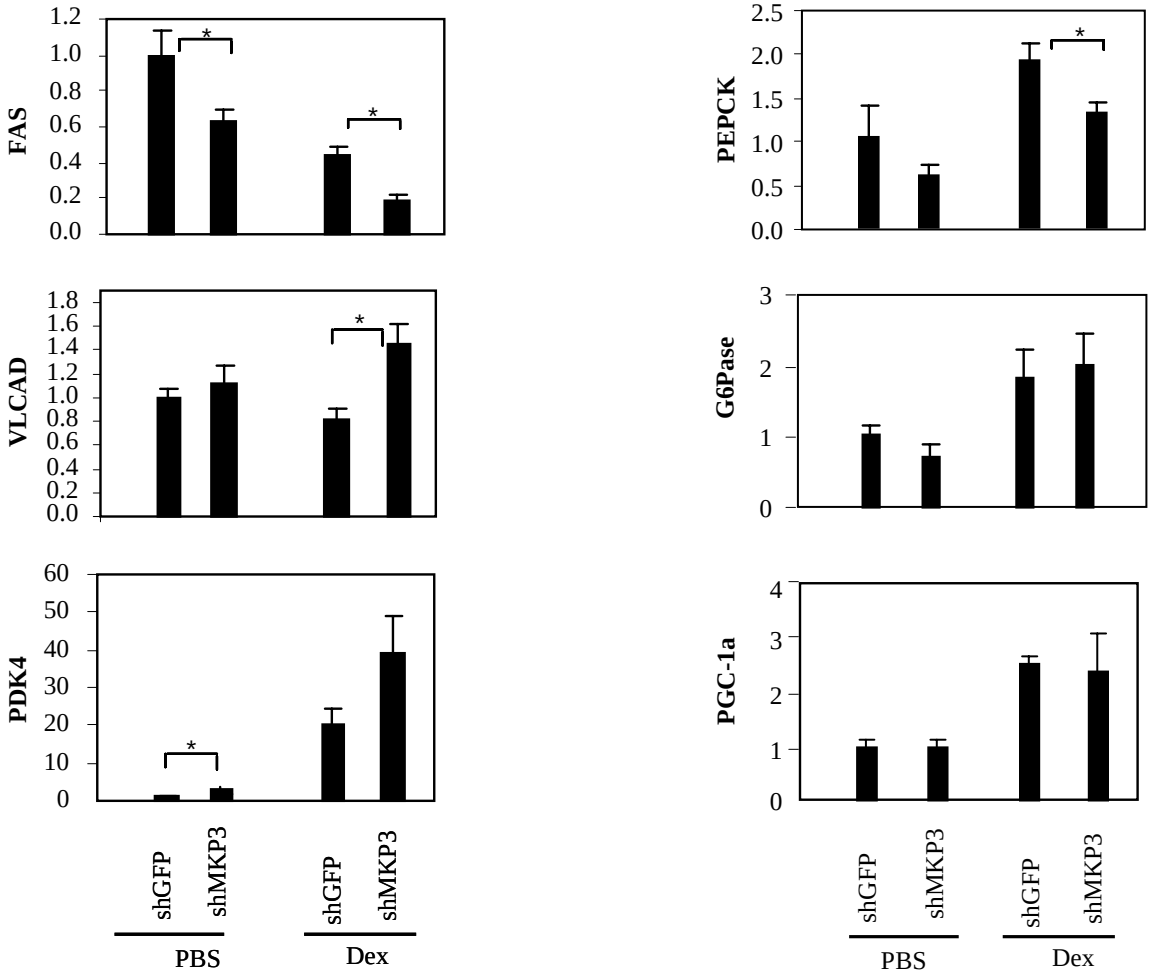
To study the regulation of MKP-3 protein by hormones, glucagon was injected into lean mice fasted for 3 hours at the dose of 1mg/kg i.p. and livers were collected 1, 5 and 20 hours post injection; insulin was injected into lean mice fasted for 6 hours at the dose of 0.5U/kg, and livers were collected 1, 5 and 20 hours post injection. Male *ob/ob* mice were purchased from The Jackson Laboratory at 7 weeks of age and fed on a chow diet with 5% calories derived from fat. After one week of acclimation, *ob/ob* mice were randomized into two groups with equal body weight and postprandial blood glucose levels. Adenoviruses expressing shGFP or shMKP-3 were injected at the dose of $3x10^9$ pfu/mouse via tail vein. Mice were sacrificed in fasted condition for plasma and tissue collection.



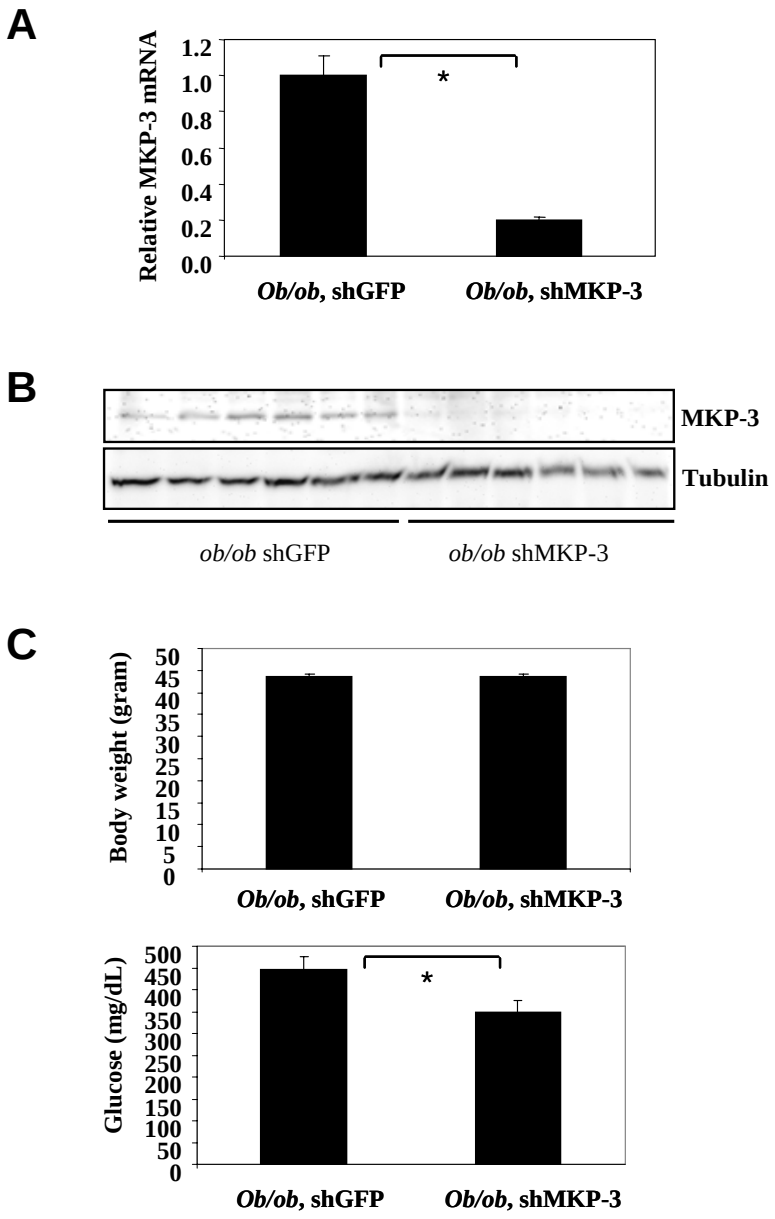
Supplemental Figure 1. Regulation of MKP-3 protein by glucagon and insulin. A. Effect of glucagon on expression of MKP-3 protein in the liver of lean mice (n=4 each group). **B.** Effect Of insulin on expression of MKP-3 protein in the liver of lean mice (n=3-4 each group). *P<0.05, hormone treated group vs. saline treated group.



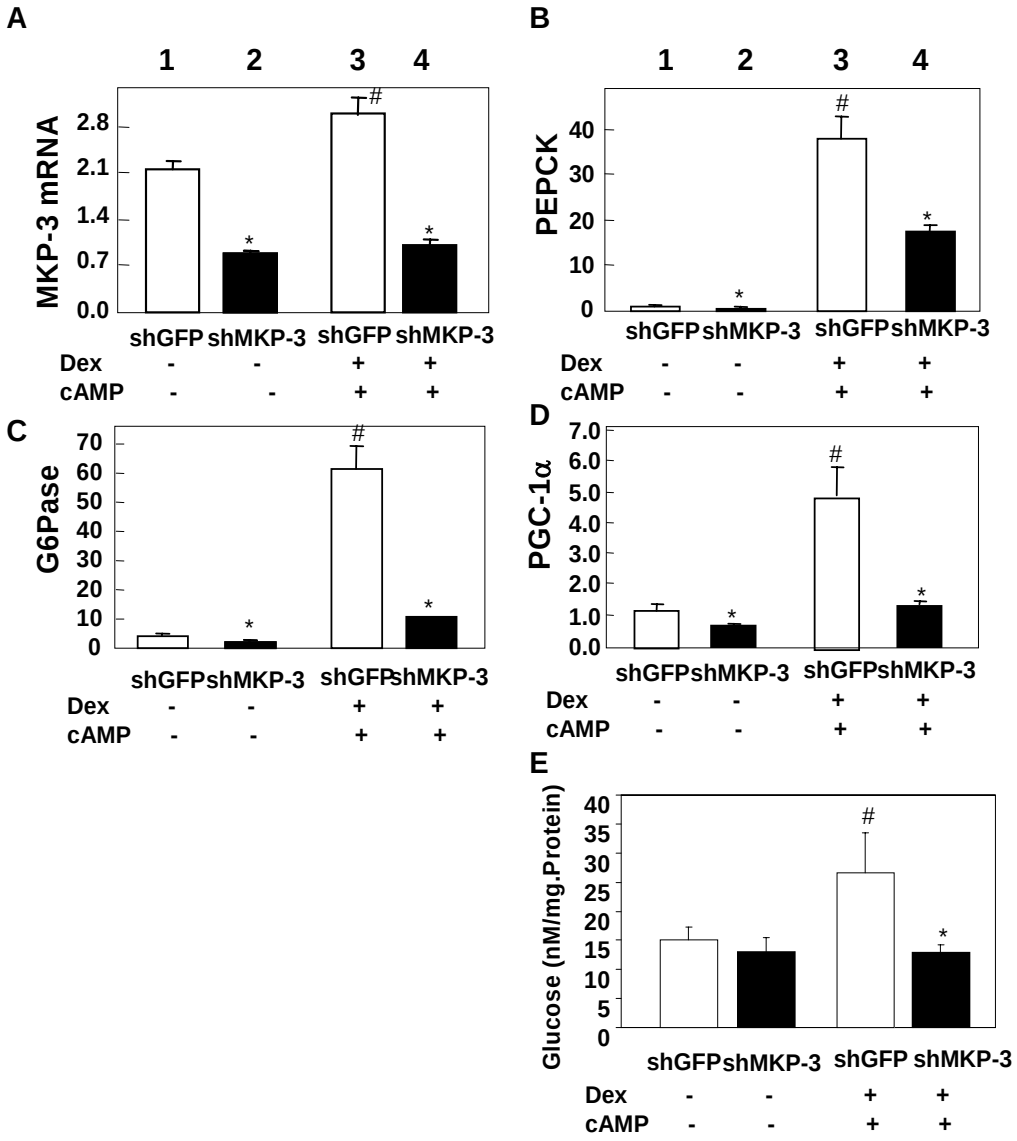
Supplemental Figure 2. MKP-3 overexpression in rat primary hepatocytes. A-E. MKP-3 overexpression in rat primary hepatocytes. MKP-3 is overexpressed in rat primary hepatocytes through adenovirus-mediated gene transfer. Expression of MKP-3 (A), PEPCK (B), G6Pase (C), and PGC-1α (D) genes as well as glucose output (E) was measured. $P<0.05$, bar 2 vs.1; 4 vs. 3. # $P<0.05$, bar 3 vs. 1.



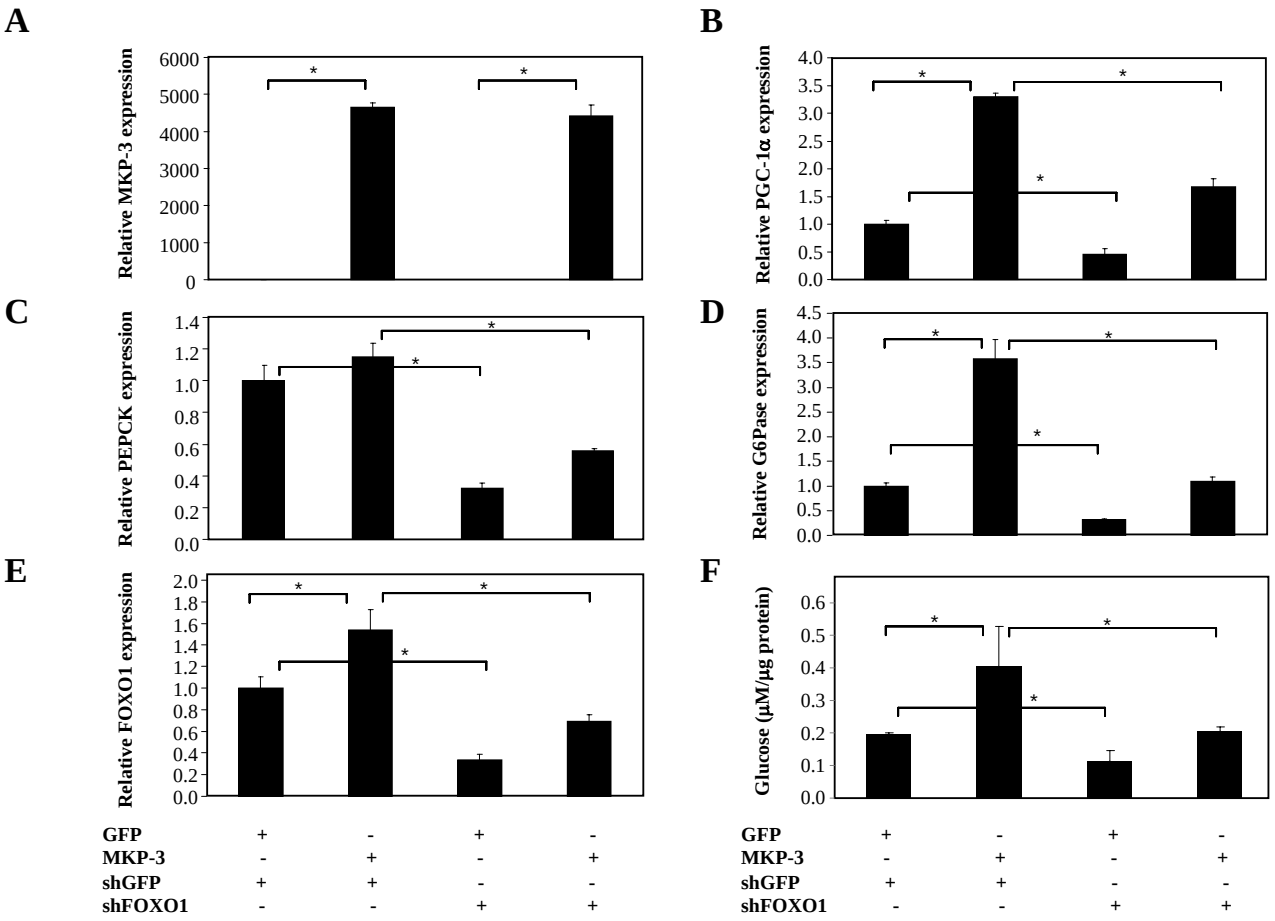
Supplemental Figure 3. Gene expression analysis in DIO mice with reduced hepatic MKP-3 expression. FAS was measured in fed state and the rest genes were measured in fasted state. *P<0.05, mice injected with Ad-shGFP vs. mice injected with Ad-shMKP-3.



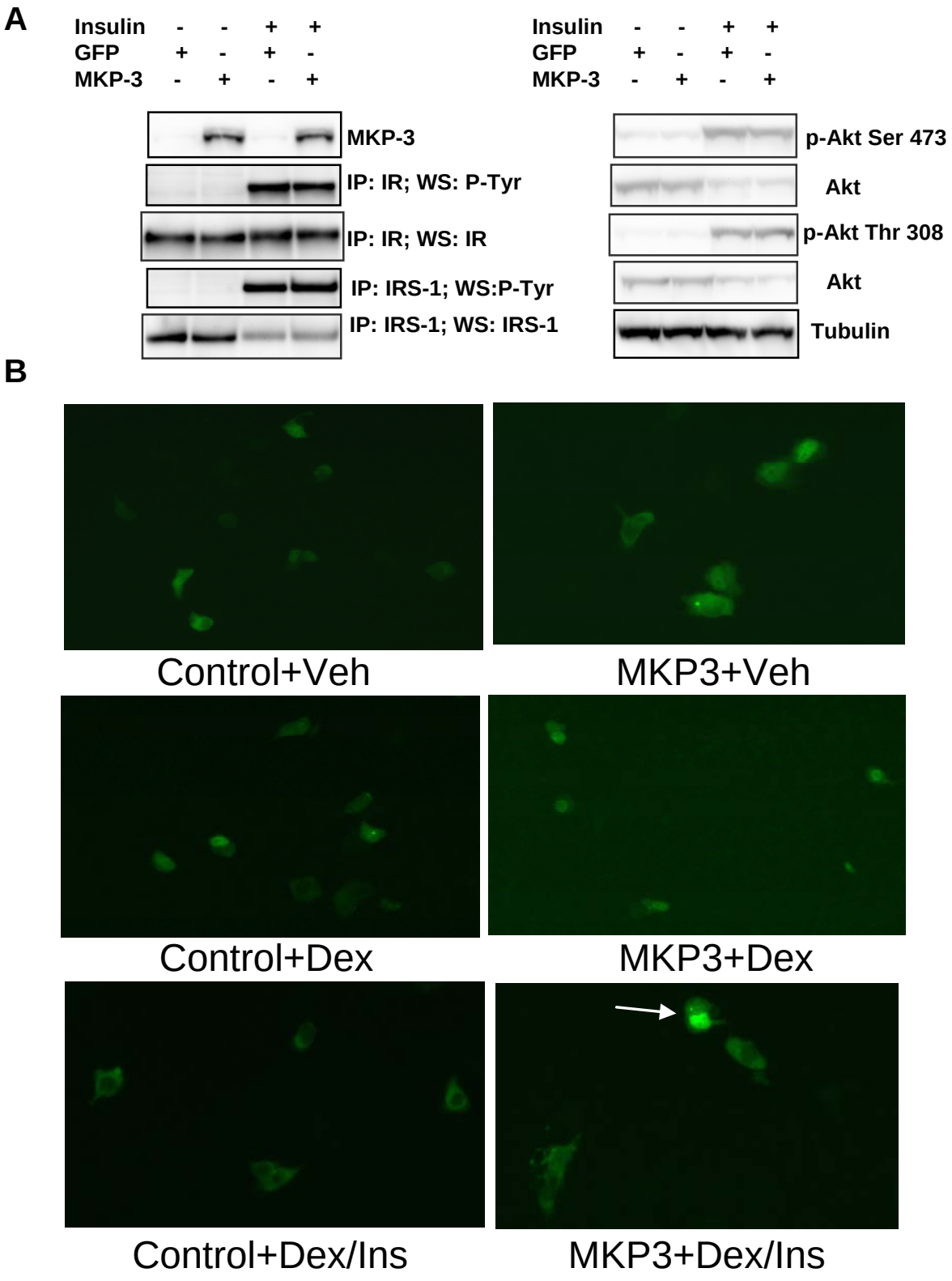
Supplemental Figure 4. MKP-3 knockdown in the liver of *ob/ob* mice. Male o*b/ob* mice in C57BL/6J background were injected with adenoviruses expressing either shGFP or shMKP-3 at the dose of $3x10^9$ pfu/mouse. Body weight and blood glucose levels were measured in fasted state. Liver samples were collected on the same day in fasted state for determination of MKP-3 mRNA and protein levels. **A.** Relative MKP-3 mRNA level (n=6 each group); **B.** MKP-3 protein expression (n=6 each group); **C.** Body weight and glucose levels (n=14-16 each group). *, $P<0.05$, mice injected with Ad-shGFP versus mice injected with Ad-shMKP-3.



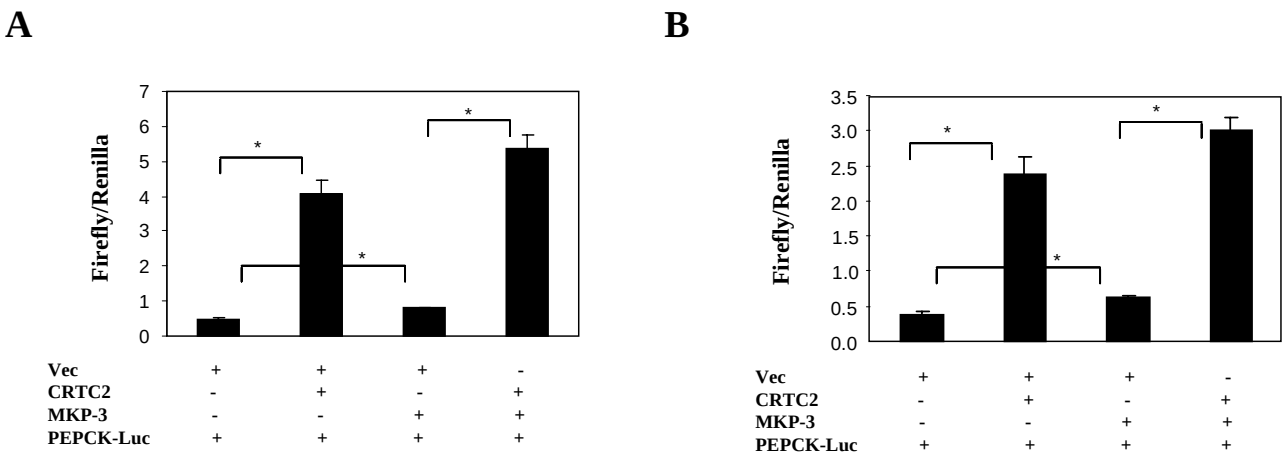
Supplemental Figure 5. MKP-3 knockdown in rat primary hepatocytes. MKP-3 is knocked down in rat primary hepatocytes through adenovirus-mediated expression of a short hairpin interfering RNA against MKP-3. Expression of MKP-3 (A), PEPCK (B), G6Pase (C), and PGC-1α (D) genes as well as glucose (E) output was measured. Dex, Dexamethasone. * P<0.05, bar 2 vs.1; 4 vs. 3. # P<0.05, bar 3 vs. 1.



Supplemental Figure 6. Effect of FOXO1 knockdown on MKP-3 stimulated glucose production. A. Relative MKP-3 mRNA levels in rat primary hepatocytes infected with Ad-shGFP or Ad-shMKP-3 together with Ad-shScramble or Ad-shFOXO1; **B-E.** Relative PGC-1α, PEPCK, G6Pase and FOXO1 mRNA levels in the same cells as described in A; **F.** Glucose production in the same cells as described in A. *P<0.05.



Supplemental Figure 7. Effect of MKP-3 on insulin signaling and FOXO1 nuclear translocation. A. Effect of MKP-3 over-expression on insulin signaling. **B.** Effect of MKP-3 over-expression on FOXO1 nuclear translocation. Representative fluorescent photos of GFP-FOXO1 expressing cells under various conditions. Veh, Vehicle; Dex, dexamethasone; Ins, insulin.



Supplemental Figure 8. CRTC2 and MKP-3 on transcription of gluconeogenic genes. A. CRTC2 and MKP-3 do not have additive effect on transcription of PEPCK promoter. **B.** CRTC2 and MKP-3 do not have additive effect on transcription of G6Pase promoter. ***P**<0.05 as indicated.